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Wastewater treatment for nutrient recovery with Ecuadorian native microalgae

María Belén Benítez Vallejo

Valeria Ochoa-Herrera, Ph.D, Directora de Tesis

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HOJA DE APROBACIÓN DE TESIS

Wastewater treatment for nutrient recovery with Ecuadorian native microalgae

María Belén Benítez Vallejo

Valeria Ochoa-Herrera, Ph.D.,
Directora de Tesis
Miembro del Comité de Tesis …….

Ródny Peñafiel, Dr. Ing.,
Miembro del Comité de Tesis ……...……..

Andrés Torres, Ph.D.,
Miembro del Comité de Tesis …...……..……

Ximena Córdova, Ph.D.,
Decana de la Escuela de Ingeniería
Colegio de Ciencias e Ingeniería..……...……...……..……

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Nombre: María Belén Benítez

C. I.: 171438745-1

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Resumen

La generación y descarga de aguas residuales son un problema a nivel mundial, especialmente en países en vías de desarrollo como Ecuador donde la mayoría de las aguas no tratadas son descargadas directamente en cuerpos de agua, ocasionando impactos en los ecosistemas y poniendo en riesgo la salud pública. El objetivo de este proyecto es estudiar la factibilidad de utilizar microalgas para la remoción de nitrógeno y fósforo como un tratamiento potencial en el Ecuador. Ensayos batch aireados y no aireados se llevaron a cabo utilizando una agua residual sintética que simule la composición de las aguas residuales de Quito con el fin de determinar la eficiencia de remoción de nitrógeno y fósforo de una cepa nativa *Chlorella sp*. Las concentraciones de NH_4-N fueron 79.9 y 83.7 mg L^{-1}, mientras que las concentraciones de PO4-P fueron 13.3 y 11.7 mg L^{-1} en bioensayos no aireados y aireados respectivamente. Manteniendo una relación N/P alrededor de 6.3 a 7.3 en el agua residual sintética. Los experimentos se realizaron a temperatura ambiente con fotoperiodos de 12 horas de luz artificial. Los resultados experimentales indican que el cultivo de algas puede eliminar exitosamente el nitrógeno y fósforo. Las eficiencia de remoción obtenidas fueron 52.6% y 55.6% para NH_4-N, 67.0% y 20.4% para PO_4-P y la producción de NO3-N fue 87.0% y 93.1% en los bioensayos no aireados y aireados respectivamente. Por tanto, la cepa nativa de *Chlorella sp.* puede ser un tratamiento potencialmente utilizado para la remoción de nutrientes en el país.

Palabras claves: microalgas, aguas residuales, remoción, amonio, nitrato, fósforo.

Abstract

The generation and discharge of wastewater are of major concern worldwide, especially in developing countries like Ecuador where the majority of untreated domestic wastewaters are directly discharged into bodies of water, resulting in severe impacts to receiving ecosystems and posing a risk to public health. The aim of this project is to study the feasibility of utilizing microalgae for the removal of nitrogen and phosphorus as a potential wastewater treatment process in Ecuador. Non-aerated and aerated batch experiments were carried out with a synthetic wastewater sample in order to determine removal efficiencies of nitrogen and phosphorus by a native strain, *Chlorella sp.* NH_4-N concentrations were 79.9 and 83.7 mg L^{-1} while PO_4-P concentrations were 13.3 and 11.7 mg L^{-1} by keeping N/P ratio around 6.3-7.3 in the synthetic wastewater. The experiments were performed at room temperature with 12 hours photoperiods of artificial light. Experimental results indicated that algae culture could successfully remove nitrogen and phosphorus. NH_4-N removal efficiencies were 52.6% and 55.6%, PO_4-P removal efficiencies were 67.0% and 20.4% and NO_3-N production were 87.0% and 93.1% in non-aerated and aerated bioassays respectively. Hence, native strain *Chlorella sp.* could be a potential treatment for nutrient recovery in Ecuador.

Keywords: microalgae, wastewater, removal, ammonium, nitrate, phosphorus.

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Introduction

In Latin America, the majority of wastewater does not receive treatment. According to the United Nations Water Statistics, in developing countries, 90% of the domestic streams are discharged directly into rivers, lakes and coastal zones without any treatment; and Ecuador is not an exception (United Nations Statistic Division, 2011). Quito is the capital city of Ecuador with a population of 2 239 191 people according to the last census conducted in 2010 (INEC, 2013). Surprisingly, Quito does not have a Wastewater Treatment Plant (WWTP) and, currently, all domestic effluents are being discharged directly into Machángara River and Monjas River without prior treatment (EPMAPS, n.d.). Ecuador is one of the richest countries in hydric resources in South America, it provides 43 000 m^3 per person per year, which is 2.5 times more than the world's mean (Cabrera *et al.*, n.d). However, it is estimated that in Ecuador, 70% of the hydrographic watershed below 2800 meters above sea level is not adequate for direct human consumption due to the presence of pathogens, solid waste contamination, hydrocarbons and other organic contaminants. Moreover, more than 80% of the industrial, agro-industrial and utilities companies generate wastewater with high organic loads that are straight discharged to the sewer systems or waterways (Cabrera *et al.*, n.d.). The discharge of untreated wastewater into water bodies has several detrimental effects on receiving ecosystems and could pose a risk to public health (Aslan and Kapdan, 2006; Taylor and Yahner, 1996).

According to Secretaría Pública Metropolitana de Agua Potable y Saneamiento (EPMAPS) Quito has potable water coverage of 99.82% for urban areas and 94.94% for rural areas from which 96.59% in urban areas and 81.97% in rural areas have sewer services (Empresa Pública Metropolitana de Agua Potable y Saneamiento, 2011). The amount of water that the city consumes each day is on average 639 million L, equivalent to a

consumption of 7400 L s^{-1}. In Quito there is no wastewater facilities, which means that all the water used in the city is discharged directly to the rivers. In fact, 81% of the rivers´ contamination comes from domestic wastewater and the remaining 19% comes from industrial discharges (Empresa Pública Metropolitana de Agua Potable y Saneamiento, 2011).

Wastewater treatment systems have generally not been implemented in Ecuador for the treatment of municipal effluents with a few exceptions in cities such as: Cuenca, Manta, Babahoyo and San Cristobal in Galapagos. In fact, the two biggest cities, Quito and Guayaquil are still discharging untreated domestic effluents into water bodies. In the case of Quito, the wastewater is mainly discharged into the Machángara river and it is estimated that almost 70% of the river's flow corresponds to untreated wastewater (Llanos, 2009). Furthermore, to date, there have been no studies in the literature on the quality of the rivers in Ecuador. Presumably rivers are contaminated because they receive discharges of domestic wastewater, however, to the best of our understanding, no studies have been published that could confirm that this is the case. In fact, in Ecuador approximately just 8% of sewage have some level of treatment (Cabrera, *et al.*, n.d.) causing in 2013, 654 555 cases of people suffering diseases transmitted by water (Dirección Nacional de Vigilancia Epidemiológica, 2013).

In wastewater treatment systems, the removal of nutrients, mainly dissolved nitrogen and phosphorus, is becoming an important step of treatment. Nutrient concentrations present in untreated wastewater can cause eutrophication in water bodies, which is the growth of unwanted plants such as algae and aquatic macrophytes (Abdel-Raouf *et al*., 2012). Eutrophication can adversely and even irreversibly affect ecosystems primarily because of the presence of high nitrogen and phosphorus concentrations (Abdelaziz *et al*, 2014). Other consequences of the presence of nitrogen compounds in the effluents are toxicity of non-

ionized ammonia to fish and other aquatic organisms, interference with free chlorine residual required in disinfection process and methemoglobinemia (reduced ability of the red blood cell to release oxygen to tissues) in influents due to excessive nitrate concentrations (above 45 g m^{-3}) in drinking water (Abdel-Raouf *et al*., 2012).

Numerous studies have demonstrated that microalgal systems hold a great potential for the removal on nitrogen and phosphorous from wastewater (Abdelaziz *et al*., 2014; Dickinson *et al*., 2013). For example, various species of *Chlorella* and *Scenedesmus* can provide very high (>80%) removals of ammonia, nitrate and total phosphorus from secondary wastewaters (Pittman *et al.*, 2011). Similarly, the nutrient removals can reach as much as 90% of the nutrients load reduction by the time the cultures reach the stationary phase (McGinn *et al.*, 2012). The concentration of the nutrient in the waste stream will govern the removal efficiency, which will define the optimization between algal strain and wastewater composition. Microalgal used to remove nitrogen and phosphorus has numerous benefits, foremost, the potential for reducing eutrophication. The nitrogen and phosphorus recovered can be recycled into algal biomass which is subsequently suitable for biofuels and fertilizer production (McGinn *et al.*, 2012). Additionally, the amounts of freshwater and commercial fertilizers needed for microalgal cultivation can be significantly reduced (Abdelaziz *et al.*, 2014). Furthermore, the benefits of using algal include lower operating costs, the discharge of oxygenated effluent into receiving water bodies (Aslan and Kapdan, 2006), and it does not generate additional waste streams that require further treatment like sludge (Pittman *et al.*, 2011).

Microalgal domestic WWT systems hold a great potential for the recovery of nutrients and the production of biofuels. Biofuels are high-volume, low-value products and this places a number of critical restraints on the use of algal for practical biofuels production. Large volumes of water and significant amounts of macro- (N, P) and micronutrients are

required and would greatly increase the cost of the process. The use of wastewater to cultivate strains is one solution to these challenges and Ecuador has an ideal climate for implementing an algal wastewater system. A low operational and maintenance cost and low-energy process for the treatment of wastewater will be developed, addressing one of the most important environmental issues in the country.

The objective of this research is to evaluate the feasibility of utilizing microalgal for nutrient recovery as a potential wastewater treatment process in Ecuador. First, domestic wastewater generated in Quito will be characterized physic-chemically. Considering the nutrients' load of the characterization a synthetic wastewater will be used to evaluate the native microalgal species for nutrient recovery in aerated and non-aerated batch bioassays.

Materials and Methods

Chemicals

Calcium chloride dihydrate, sodium nitrate, monopotassium phosphate, ferrous sulfate, zinc sulfate heptahydrate, cupric sulfate pentahydrate, manganous sulfate monohydrate, glucose, boric acid were obtained from Reactivos H.V.O (Quito, Ecuador). Magnesium sulfate heptahydrate and ammonium carbonate were purchased from Representaciones Vamarth (Quito, Ecuador).

Domestic wastewater samples

Domestic wastewater samples were taken from six different discharge points located in the south part of Quito, Ecuador in October of 2014. These points were assigned by the local publicly owned water company (EPMAPS) in Quito; Table 1 presents general information about sample sites.

Microalgae Strain

The native microalgae was kindly donated from a *Chlorella sp.* strain from "ESPE", Escuela Politécnica del Ejército. Microalgae was cultivated in a tubular photobioreactor (PBR) with inlet upward airflow. The PBR has a 10 L capacity, however it remains at 8 L and it is illuminated with fluorescent light with 12 hours photoperiods.

Synthetic domestic wastewater

The composition of the synthetic domestic wastewater was (in mg L^{-1}): $CaCl_2.2H_2O$ (37.4), $MgSO_4.7H_2O$ (56.7), $(NH_4)_2CO_3$ (297.9), $NaNO_3$ (6.8), KH_2PO_4 (55.8), $FeSO_4.7H_2O$ (0.3), $ZnCl_2$ (0.1), $CuSO_4.5H_2O$ (0.2), $C_6H_{12}O_6$ (9.3) y H_3BO_3 (0.001). The solution was sterilized in the autoclave during 15 minutes at 121 ºC prior bioassays.

Nutrient removal bioassays

The concentrations of nutrients in the removal bioassays were 86.7, 1.1 and 12.7 mg L^{-1} of NH_4^+-N, NO_3^- -N and PO_4^{3-}-P respectively. Batch bioassays were conducted in duplicates using 1000 mL flasks supplied with 1000 mL of synthetic wastewater containing the nutrients and 8 mL of microalgae. Abiotic controls (lacking microalgae) were run in parallel to correct for the possible removal of nutrients by abiotic reaction. Killed-microalgae control (by autoclaving at 120ºC for 15 min) were also set up to determine background nutrient concentration and to quantify the compounds removal by sorption to the algal biomass, respectively. All bioassays were incubated in an orbital shaker (MAX-Q 2508 Barnstead LabLine, USA) at 100 rpm at room temperature (23±2ºC). The bioassays were illuminated by tubular fluorescent lamps (OSRAM, 20W) with 12 hours photoperiods for 17 days. For the aerated bioassays a system of aquarium pumps was incorporated, which provided an air flux of 1 L s^- to each flask.

Cell density

Cell density was measured with a Neubauer counting chamber. The sample was prepared by diluting 40 μL of sample in 160 μL of lugol's solution, in order to immobilize the cells. The counting chamber was loaded with 10 μL of the mixture. The counting was done in the 40x lens of a Leica CME microscope. The cell density was calculated using the Eq. 1.

$$Cell\ density\ [\#\ cells\ mL^{-1}] = Average\ cells\ \#\ \times dilution\ factor\ \times 10^4\ (\boldsymbol{Eq.\ 1})$$

Biomass and lipids extraction

Algal biomass and lipid extraction were measured at the beginning and the end of the removal bioassays. In the case of biomass determination, a 45 mL sample was centrifuged at 5000 rpm for 10 minutes and dried at 105℃ for 12 hours. The biomass was determined by weight difference. A solvent extraction method was used to obtain the lipids from the microalgae. The dry biomass was used and it was grinded until it looked as powder. The biomass was transferred to 15 mL falcon tubes; 2 mL of chloroform ($CHCl_3$) and 1 mL of methanol (CH_3OH) were added and centrifuged at 5000 rpm for 10 minutes. The supernatant was transfer to another falcon tube, 5 mL of distillated water were added and it was centrifuged. Next, the third layer containing a mix of chloroform and lipids was transferred to digestion tubes, previously weighed. This process was repeated for three times until obtaining a clear supernatant. Finally, when the lipids were completely dry the tubes were weighed and the dry lipids and lipids content were calculated (Eq. 2 and 3).

$$Dry\ lipids\ (g) = glass\ tube\ with\ lipids\ - glass\ tube\ (\boldsymbol{Eq.\ 2})$$

$$Lipidis\ content\ \%\ \left(\frac{w}{w}\right) = \frac{Dry\ lipids\ (g)}{Dry\ biomass\ (g)} \times 100\ (\boldsymbol{Eq.\ 3})$$

Analytical Methods

Turbidity, potential redox (ORP), pH, dissolved oxygen (DO), temperature and conductivity (SM 2510) were measured with a Thermo Scientific Orion 5-Star portable multiparameter meter (Thermo Scientific, Beverly, MA 01915, USA). Ammonium, nitrate, chloride and fluorides were measured using an Orion ion-selective electrode respectively. Sulfides, chemical oxygen demand (COD) and phosphates were measured by a colorimetric method using a Spectronic 20D+ spectrophotometer (Thermo Fisher Scientific Inc. Waltham, MA, USA). Sulphates and total solids (TS) were measured by a gravimetric method.

Parameters such pH, DO, temperature, ORP and conductivity were daily monitored. Spending a day, 90 mL samples withdrawn from flasks were centrifuged at 5000 rpm for 10 minutes to separate algae. NH_4^+–N, NO_3^--N and PO_4^{3-}-P measurements were carried out in clear supernatant by electro analytical methods for nitrate and ammonium and vanadate-molybdate colorimetric method for phosphate. All analytic methods were determined according to Standard Methods for Examination of Water and Wastewater (APHA, 2012).

Results and Discussion

Domestic wastewater characterization

The physical-chemical characterization of six discharge points of domestic wastewater samples from Quito, Ecuador is presented in Table 1. All the wastewater samples were neutral with a pH of around 7. Similarly, redox potential was about 360-400 mV. Temperature and DO varied in each discharge point, from 1 to 3.6 mg L^{-1} and 16 to 19 ºC respectively with an exception, "Ortega" discharge point had a higher OD level (5.9 mg L^{-1}) and lower temperature (10ºC). As expected at higher temperatures the DO was lower. Conductivity was around 650-850 μS cm^{-1}, once again Ortega was an exception with a value of 233 μS cm^{-1}. Fluoride and sulphides concentrations were very low in all the samples from

0.1 to 0.3 mg L^{-1} and from 9 to 13 mg L^{-1} respectively. Sulphates concentration ranged from 70 to 100 mg L^{-1}. Nutrients concentration, nitrate, ammonium and phosphate, varied in all the points from 3 to 10 mg L^{-1}, 13 to 36 mg L^{-1} and 13 to 28 mg L^{-1} respectively. Chloride concentration was also different, the lower value was 7 mg L^{-1} and the highest was 119 mg L^{-1}. Finally, TS and COD were different in all the samples from 250 to 1060 mg L^{-1} and 30 to 895 mg L^{-1} receptively. As expected, a correlation between TS and COD was observed.

In general terms, wastewater samples present different characteristics, which doesn't allow determining a typical domestic wastewater composition of the city. According to EPMAPS, these differences show the possibility of infiltration in the system and the mix of industrial and domestic wastewater effluents. However, for this study wastewater characterization from the discharge point named "Calicanto" was chosen to prepare the synthetic wastewater. Calicanto sample presented typical values for medium to high strength domestic wastewater, for instance, the COD was 895.6±36.4 mg L^{-1}, while the typical COD range is 500 to 800 mg L^{-1} (Metcalf and Eddy, 2014). In the case of the contaminants of interested, typical domestic wastewater values for ammonium concentrations are in a range of 45-75 mg NH_4^+ L^{-1} (Henze and Comeau, 2008), 6-24 mg PO_4^{3-} L^{-1} (Minnis, n.d.), 10-15 mg P L^{-1} (Henze and Comeau, 2008) and less than 1 mg NO_3^- L^{-1} (Minnis, n.d.). Calicanto wastewater sample, showed concentrations of 36±0.6 mg NH_4^+ L^{-1}, 28.4±0.3 mg PO_4^{3-} L^{-1}, 9.3±0.1 mg P L^{-1} 3.6±0.0 mg NO_3^- L^{-1}.

NH_4-N and PO_4-P removal

The variation of NH_4-N concentration with time for 17 days of non-aerated batch operation is depicted in Fig. 2B. NH_4^+-N removal efficiency was 52.6±5.9% when the initial concentration of the synthetic wastewater was 79.9±3.0 mg L^{-1}. The variation of NH_4-N concentration of aerated batch operation is represented in Fig 3B, with a removal efficiency

of 55.6±7.4% when the initial concentration was 83.7±0.7 mg L^{-1}. The ammonium removal efficiencies achieved in this study were almost the same to some of other studies. For example, a 50% NH_4-N removal was observed when the media concentration was between 41.8–92.8 mg L^{-1} in aerated batch bioassays (Aslan and Kapdan, 2006); which is the same range of the initial concentration of the synthetic wastewater employed in this study. In other studies with *Chlorella sorokiniana*, ammonium concentrations were reduced almost by 65%, however the initial concentration was 35-40 mg L^{-1} (Lizzul *et al*., 2014), which is almost half of the concentration of this study. Removal efficiencies for *Chlorella sp.* were 82.4% from a wastewater before primary settling with a initial concentration of 33.4 ± 0.6 mg L^{-1}, 74.7% from a wastewater after primary settling with a initial concentration of 32.2±0.4 mg L^{-1} and 78.3% from a centrate from sludge centrifuge with a concentration of 71.8±1.1 mg L^{-1} (Wang, 2010).

Chlorella and *Scenedesmus* species can grow in a variety of organic and inorganic compounds; in fact they can change from autotrophic to heterotrophic when the carbon source change (Larsdotter, 2006). The two main nitrogen sources for *Chlorella* growth are ammonium and nitrate salts. When both elements are supplied together, microalgae will preferentially assimilate ammonium first and incorporated it into their organic compounds (De-Bashan *et al.*,2005). As a result, if ammonium is present any other nitrogen source won't be assimilated (Larsdotter, 2006). Other findings show that species as *Chlorella sorokiniana* prefer ammonium as a source of nitrogen, this conforms to a metabolic preference for reduced nitrogen species that is common within many types of algae (Lizzul *et al*., 2014).

The removal efficiencies for PO_4-P were 67.01±1.5% and 20.4±6.9% when the initial concentration was 13.3±0.2 mg L^{-1} and 11.7±0.6 mg L^{-1} in non-aerated and aerated batch conditions respectively (Fig 2C and Fig 3C). In the case of the phosphate removal in non-

aerated batch operation, the efficiency was similar to a study with *Chlorella vulgaris*, which had an initial concentration of 7.7 mg PO_4-P L^{-1} and a removal efficiency of 78% (Aslan and Kapdan, 2006). However, the phosphate removal efficiency of aerated batch operation is similar to the study with *Chlorella kessleri,* which was able to uptake between 8 and 20% phosphorus under the light/dark cycle for PO_4-P concentration of 10 mg L^{-1} (Lee and Lee, 2001), initial concentration that is very similar to the one of this study.

The ways in which phosphorus can be removed form wastewater are direct cellular absorption under aerobic conditions and sedimentation under anoxic conditions (González *et al.*, 1997). In the case of this study, phosphorus was removed in aerated conditions by the interaction with nitrogen, because of the absence of a sedimentation zone that didn't permit a phosphorus precipitation. Considering nitrogen is the limiting nutrient in the synthetic wastewater, phosphorus concentration will continue to be high after ammonium exhaustion (González *et al.*, 1997). The maximum NH_4-N removal occurred at 192 h (Fig. 1B), when the concentration was 39.9 mg L^{-1}, thus PO_4-P removal started at 240 h (Fig. 1B).

The removal rates were 0.12 mg NH_4-N L^{-1} h^{-1} and 0.03 mg PO_4-P L^{-1} h^{-1} in non-aerated bioassays, while in aerated conditions were 0.34 mg NH_4-N L^{-1} h^{-1} and 0.03 mg PO_4-P L^{-1} h^{-1}. The NH_4-N removal efficiency between the non-aerated and aerated bioassays was almost the same because it occurred by the 192 h period, however the PO_4-P removal efficiency decreased by a factor of 3.4 in the aerated bioassays. Aeration can damage the cells when the bubbles detach from the sparger, breakup, collide or burst in the liquid's surface (Acién Fernández *et al.*, 2013), this could directly influence in a reduction in PO_4-P removal because in aerated bioassays PO_4-P removal took longer than in non-aerated bioassays.

The optimal inorganic N/P ratio for freshwater algae growth was suggested to be in the range of 6.8–10 (Wang, 2010). In the case of non-aerated bioassays the ratio was 6.32 and in aerated conditions was 7.28. As a result, the N/P ratio was good enough for the operation of

both experiments.

NO_3-N production

The biological depletion of NH_4^+-N produced an increment in NO_3-N in the synthetic wastewater bioassays. The nitrogen conversion could possible happen by non-biological mechanisms such as air-stripping, absorption and sedimentation (González *et al.*, 1997). In this study two aerobic batch operation systems were evaluated, considering the aerated batch condition, air-stripping mechanism could be responsible for the nitrogen conversion. However, one of the conditions required for air stripping is a pH of around 9, yet the nutrient removal bioassay had a pH of 6.1-6.2. Hence, air stripping wasn't a nitrogen removal mechanism during this study.

It is very likely that a nitrification process was carried by the microalgae since a NO_3-N production was observed. Fig 2A and 3A show the increase in NO_3-N concentration over the 17 days of operation. The productions were 87.0±0.2% and 93.1±0.0% when the levels increased from 3.9±0.0 to 30.1±0.6 mg L^{-1} and 1.4±0.0 to 19.6±0.2 mg L^{-1} in non-aerated and aerated batch conditions respectively. The production rates were 0.16 and 0.04 mg NO_3-N L^{-1} h^{-1} in non-aerated and aerated bioassays. Studies with Chlorella vulgaris cultures also showed increments in nitrate levels from 5 to 45 mg L^{-1} after 144 h of operation (González et al., 1997).

In non-aerated bioassays the NO_3-N was produced since 144 h (Fig. 2A) despite NH_4-N reduction started by the 48 h (Fig 2B); which indicates a previously nitrite production (NO_2). On the other hand, in the aerated bioassays the NO_3-N production initiated at the same time NH_4-N started been reduced (48h) (Fig 3A and 3B), indicating that as expected aeration allows a faster conversion of NH_4 to NO_3.

Nitrogen Balance

The NH_4-N removal generated a NO_3-N production (Fig. 2A and Fig. 3A), hence a nitrogen balance was performed in order to quantify the initial and final nitrogen concentration. In non-aerated batch operation the initial N-concentration was 83.8±3.0 mg L^{-1} and the final was 68.1±6.8 mg L^{-1} (Table 2), with a difference of 15.7 mg L^{-1}. Similarly, in aerated batch operation the initial concentration was 85.1±0.8 while the final was 56.8±0.3 mg L^{-1} (Table 2) with a difference of 28.3 mg L^{-1}. It is expected that the differences between the initial and final concentration (Fig. 4) are due to the microalgae nitrogen assimilation, considering that the nitrogen content of algal biomass is around 5-10%, i.e. 15-30 mg-N for 300 mg dry cell weight (Lee and Lee, 2001). In this study, the final biomass concentration was 933.7 mg L^{-1} and 370.4 mg L^{-1}, which will result in 46.7 and 18.51 mg N L^{-1}, considering 5% of nitrogen content, however the difference in the nitrogen balance was 15.7 and 28.3 mg L^{-1} in non-aerated and aerated bioassays, respectively. As a result, non-aerated bioassays had an assimilation of 1.7%, while aerated had a 7.7% of nitrogen assimilation. Additionally, microalgae assimilation is expected because nitrogen containing compounds such as ATP and NADPH are produced actively when microalgae undergo photosynthesis, or when the cells are illuminated. (Lee and Lee, 2001)

Monitoring of daily parameters

The initial pH of the nutrients removal bioassays was between 8.5-8.9, in the abiotic control and heat-killed control, the pH remained constant during the 17 days of operation. However, in the nutrients removal treatment bioassays it declined until it reached values between 6.1-6.2. Ammonium was the nitrogen source in this study, despite it is a good nitrogen source for miroalgae growth in unfavorable concentrations it can cause growth inhibition or acidifys the culture media during algal growth (Karthikeyan *et al.*, 2012).

Moreover, when ammonium is used as carbon source, pH can decrease to 3 which is to acid for microalgae growth (Larsdotter, 2006). The temperature varied from 21 to 25 ºC, the optimal temperature for most of algae species is between 15 and 26 ºC reaching a maximum cell density at 23 ºC (Kumar *et al*., 2010). Dissolved oxygen (DO) varied from 4-7 mg L^{-1} in non-aerated batch operation and 3-5 mg L^{-1} in aerated batch operations. The dissolved oxygen of the study was appropriated considering that oxygen levels above air saturation, 7.2 mg O_2 L^{-1}, can inhibit photosynthesis in many algal species. Furthermore, elevated levels of oxygen combined with high levels of irradiance can lead to severe photo-oxidation that reduces the yield of the cultures (Acién Fernández *et al.*, 2013). Redox potential oscillated from 100 to 400 mV in non-aerated and 200 to 350 mV in aerated bioassays. Finally, conductivity remained practically constant in abiotic control and heat killed control with values of 410-430 µS cm-1, while in nutrients removal treatment bioassays a small reduction was observed from 461 to 380 µS cm-1. This indicates an algae nutrients reduction because the treatments conductivity decreased which shows that fewer ions ($NO3^-$ and $NH4^+$) were presented during the operation time.

Microalgae and bacteria competition

The main purpose of this study was to show that native Ecuadorian microalgae are capable of removing nutrients from a synthetic wastewater. In fact, the use of several microalgae species as tertiary water treatment was reviewed more than a decade ago and continues to be evaluated today (Gonzalez-Bashan *et al.*, 2000). The fundament assumption is that microalgae will transform the contaminants to nonhazardous materials in order to reuse or discharge the water in a safety way. Nevertheless, wastewater carries a large microbial population, meaning a possible interaction or competition between microalgae and bacteria in the wastewater bioremediation of the microalgae (Gonzalez-Bashan *et al*., 2000).

Moreover, competition for limiting nutrients such as phosphate can exist between bacteria and algae (Qu et al., 2014).

In the case of this study, inoculated microalgae and sterilized synthetic wastewater were used with the purpose of avoiding the presence of other microorganisms, yet it is possible the presence of environmental bacteria in the bioassays. However, the nutrients removal is granted to microalgae considering that the maximum removal occurred until day 17th. In fact, the relative long hydraulic retention times of microalgae detain the widespread application of the algal treatment process when comparing to conventional activated sludge process that can achieve efficient overall reduction of COD, ammonium, and phosphorus in 4– 6 h (Wang, 2010). Other studies, found that the maximum nutrient removal were achieved in 10 h by introducing bacteria into the system (Wang, 2010).

An investigation carried out with two microorganisms, *Chlorella vulgaris* and *Azospirillum brasilense* (nitrogen fixing bacteria) showed that the removal of the co-immobilization of both microorganisms was superior to the removal by the microalgae alone (De-Bashan *et al*., 2004). In 6 days, the co-immobilization reached a removal of 100% ammonium, 15% nitrate and 36% phosphorus, compared to the microalgae removal of 75% ammonium, 6% nitrate and 19% phosphorus (De-Bashan *et al*., 2004). This research demonstrate that in this study nutrients removal can be attributed to microalgae because the removal efficiencies were not as high at the ones achieved when bacteria are removing nutrients as well, nor the retention times were that short. Considering the same study it is important to highlight that bacterial presence can contribute in a positively form because nutrient absorption capacity of the microalgae increases from the association. Despite, microalgae plays an important role owing to the fact that *Azospirillum brasilense* alone did not remove measurable quantities of ammonium or phosphorus, while Chlorella species were

capable of eliminating most of the ammonium when immobilized alone (De-Bashan *et al.*, 2004). It has been observed that bacteria and microalgae exhibit mutual benefit relationship, in which bacteria profit from the exudates of *Chlorella vulgaris*, and the microalgae growth is promoted by the bacterial products such as carbon dioxide and inorganic compounds (Qu *et al.*, 2014).

Cell density

The initial cell density in non-aerated bioassays was $3.5x10^6$ cells mL^{-1} while the final was $7.3x10^6$ cells mL^{-1}. In the case of aerated batch operation the initial cell density was $1.3x10^6$ cells mL^{-1} while the final was $1.7x10^6$ cells mL^{-1} (Fig. 4). In both experiments, there was an increment in cell density, however final non-aerated bioassays cell density was much higher than aerated bioassays. This result can be explained with the fact that the aeration permitted a higher probability of contamination in the bioassays. A contamination in the batch bioassays is likely in part due to the long hydraulic retention times (> 7 days) required for nutrient removal which may have provided sufficient time for the proliferation of algal grazers (McGinn *et al.*, 2012). It is not consider that the NH_4-N and NO_3-N concentrations could influence in cell density because both experiments had almost the same concentration, however just in the aerated bioassays the cell density did not significantly incremented. A study with *Chlorella kessleri* showed that with different NO_3-N concentrations the final cell concentration was almost the same, which suggests that there is not growth inhibition caused by the nutrients concentration so other factors such as CO_2 mass transfer, light intensity or mixing may limit the microalgae growth (Lee and Lee, 2001).

Biomass concentration and lipids content

Biomass concentrations at the beginning of the bioassays were 215.6 and 105. 9 mg L^{-1}, while final concentrations were 933.7 and 370.4 mg L^{-1} in non-aerated and aerated batch operation respectively (Table 3). The increment in non-aerated was 718.1 mg L^{-1} and in aerated was 264.44 mg L^{-1}. In both cases, the biomass concentration increased over the operation time, which indicates a microalgae growth. However, under non-aerated conditions the growth was more pronounced as seen in the cell density too (Fig.3).

Concerning to lipids content the initial contents were 11.8 and 8.6%; the final contents were 16.7 and 10.8% in non-aerated and aerated batch operation respectively. The lipids content increased in 4.91% in non-aerated conditions and 2.2% in aerated batch operation. The lipids content increment is not significantly because nutrient limitation is well known to cause an increase in lipid and TAG contents or starch content in algal biomass (Dickinson et al., 2013). In fact, it is known that under partial nitrogen deprivation, microalgae grow at lower rates but produce significantly more lipids, which are reserve compounds synthesized under stress conditions, even at the expense of lower productivities (Kumar et al., 2010)

Conclusions

Nutrients removal from a synthetic wastewater using native microalgae was successfully studied in non-aerated and aerated batch bioassays. The use of an orbital shaker with no extra aeration resulted in better nutrients removal efficiencies and microalgae growth that adding aeration with pumps. Nutrients removal with microalgae was shown to be an effective alternative to treat domestic wastewater considering that NH_4-N efficiency removal was 52.6±5.9% and PO_4-P was 67.0±1.5%. It is highly recommended to study the microalgae nutrients removal in continuous system since other studies demonstrate that with this system

the overall biomass productivity is higher because the time required to accumulate biomass includes the initial 2–3 days when growth rates are high but biomass concentrations are low and the final 2–3 days when the reverse is true (McGinn *et al.*, 2012). Moreover, the hydraulic retention times are shorter that in batch operation (McGinn et al., 2012) which can assure less chance of contamination. This study contributes to the development of a wastewater treatment process that could be successfully implemented in Ecuador.

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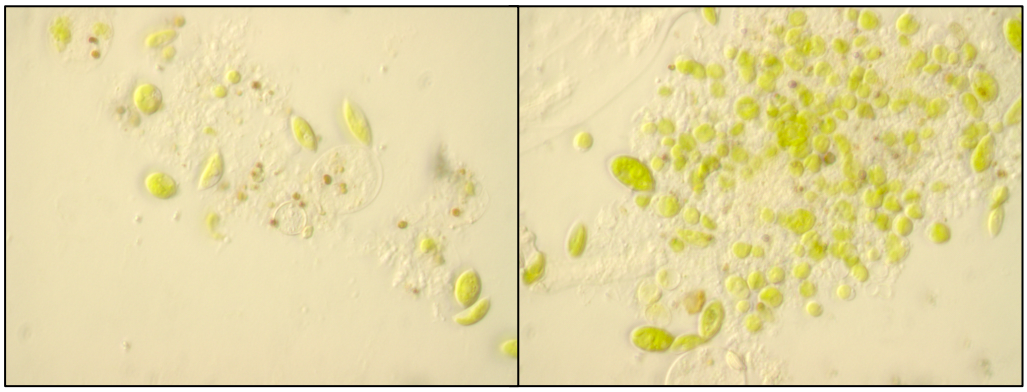

Figure 1: Native microalgae strain *Chlorella sp.*

Table 1: Description and physical-chemical characterization of domestic wastewater samples

Parameter	Unit	Pasocucho	Ortega	Calicanto	Capuli	Caupicho 2	Caupicho
Description		**Ravine**	**Collector**	**Ravine**	**Ravine**	**Collector**	**Collector**
Ammonium	**mg L^{-1}**	14.5±0.8	0.8±0.01	36±0.6	14.7±0.6	25.7±0.2	13.1±0.6
Chloride	**mg L^{-1}**	50.3±0.8	7±0.1	119.5±10.1	84.6±0.3	119,5±3.7	87.7±1.4
COD	**mg L^{-1}**	178±9.1	30.2±12.1	895.6±36.4	435±3	430.8±21.2	225.1±15.1
Conductivity	**µScm^{-1}**	849.5±4.9	233.9±4.7	808.5±72.8	674±15.6	833±1.4	663±11.3
Dissolved Oxygen	**mg L^{-1}**	1±0.0	5.9±0.2	3.6±0.6	3.5±0.2	1.7±0.5	3.4±0.2
Fluoride	**mg L^{-1}**	0.3±8.6E-4	0.1±3.5E-4	0.2±1.1E-3	0.2±6.3E-4	0.2±1.3E-3	0.2±1.3E-3
Nitrate	**mg L^{-1}**	3.7±0.0	16.3±0.8	3.6±0.0	3.2±0.3	4.8±0.02	9.8±0.4
pH	-	7.4±0.0	7.1±0.3	7.6±0.0	7.4±0.0	7.4±0.0	7.2±0.0
Phosphate	**mg L^{-1}**	3.8±0.4	1.2±0.5	28.4±0.3	6.3±0.5	6.3±0.5	3.5±0.9
Redox Potential	**mV**	406.2±1.4	403.4±1.6	397.6±5.9	368.1±5.7	359.7±1.3	362.4±0.2
Sulphates	**mg L^{-1}**	85.6±2.3	70±3.5	98±10.5	91.4±3.5	98.8±18.6	76.6±3.5
Sulphides	**mg L^{-1}**	9.1±1.4	11.7±1.1	12.7±0.3	12.8±1.4	12±0.3	11.2±0.8
Temperature	**ºC**	16.3±0.0	10.9±0.4	17.3±0.1	17.3±0.4	19.1±0.1	18±0.1
TS	**mg L^{-1}**	754±42.4	260±5.7	1066±25.5	786±127.3	722±25.5	592±33.9
Turbidity	**NTU**	38.1±0.1	5.2±0.7	289.5±6.4	72.3±2.1	55.6±3.7	34±1.3

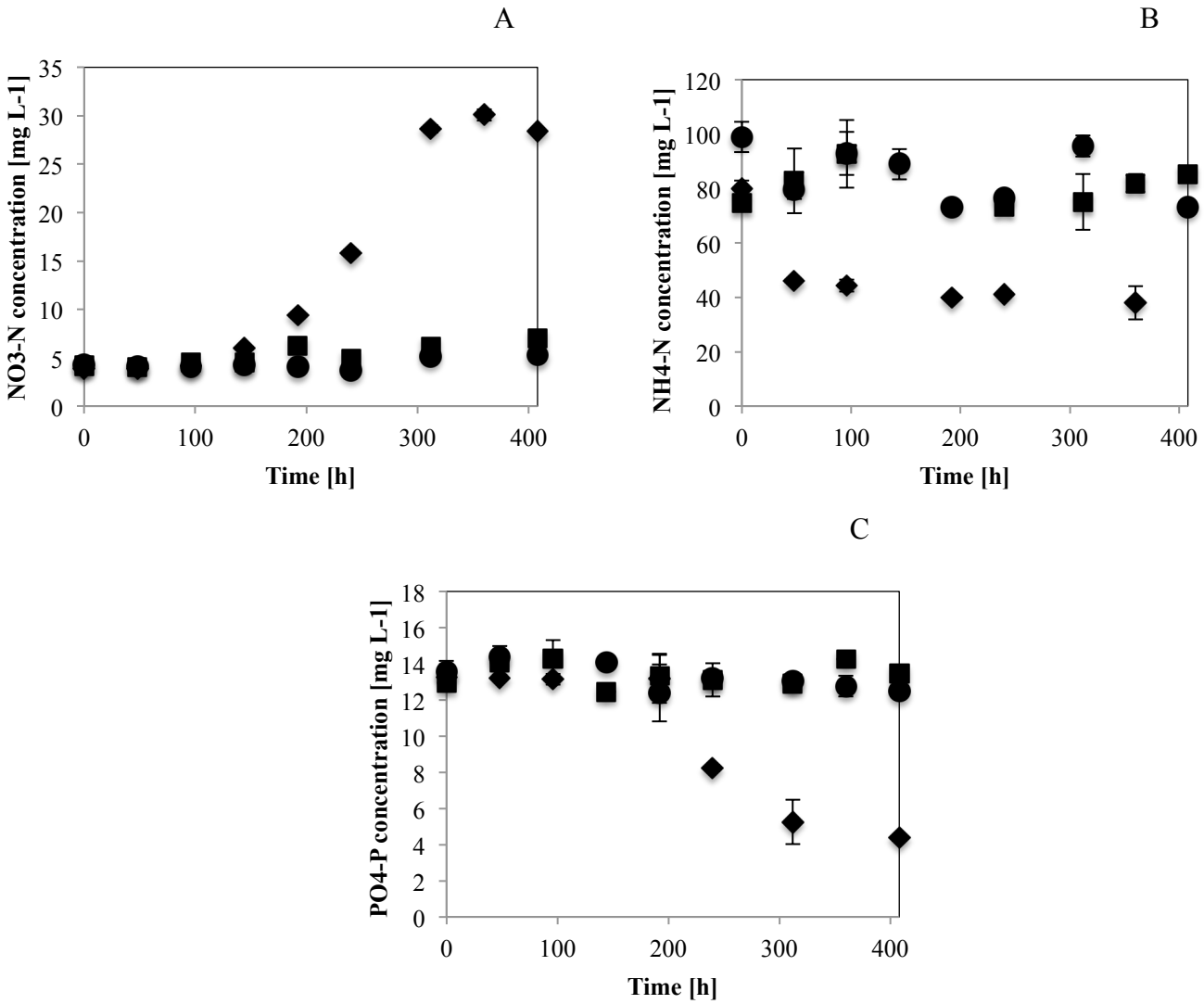

Figure 2: Nitrate production (A), ammonium removal (B) and phosphates removal (C) in non-aerated batch operation, nutrient removal treatment bioassays (◆) abiotic control bioassays (•) and heat killed control bioassays (■).Error bars (shown if larger than the symbols) represent standard deviations of duplicate assays.

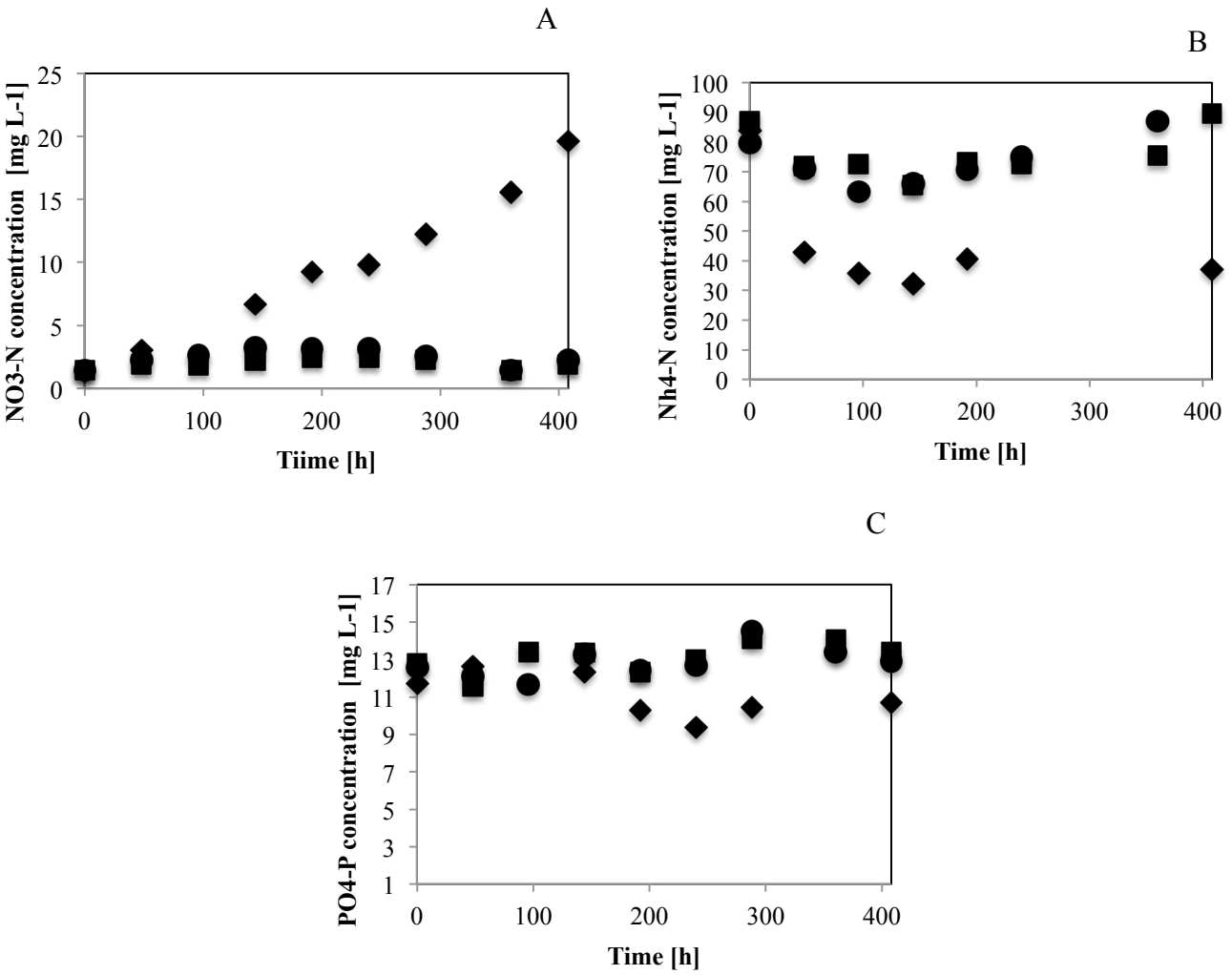

Figure 3: Nitrate production (A), ammonium removal (B) and phosphates removal (C) in aerated batch operation, nutrient removal treatment bioassays (◆) abiotic control bioassays (•) and heat killed control bioassays (■).Error bars (shown if larger than the symbols) represent standard deviations of duplicate assays.

Table 2: Nitrogen balance of aerated and non-aerated nutrients removal treatment bioassays

N-concentration	Units	Non-aerated conditions	Aerated conditions
Initial	mg L^{-1}	83.8	85.1
Final	mg L^{-1}	68.1	56.8

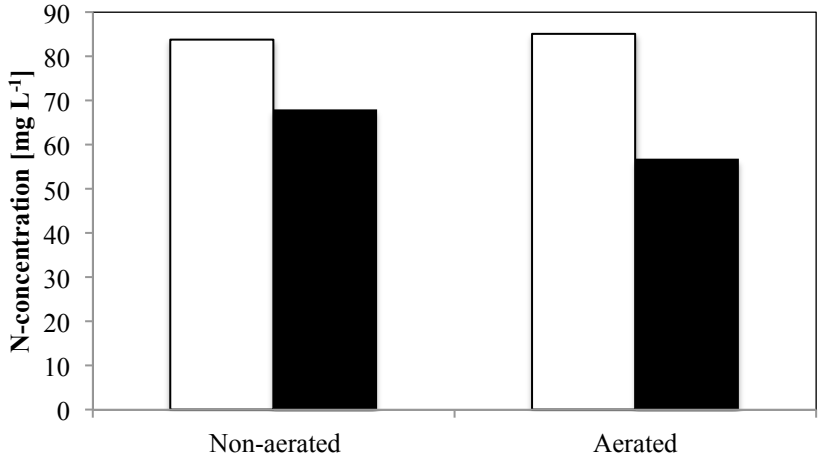

Figure 4: Nitrogen balance of aerated and non-aerated nutrients removal bioassays. Initial concentration ( ) and final concentration (■)

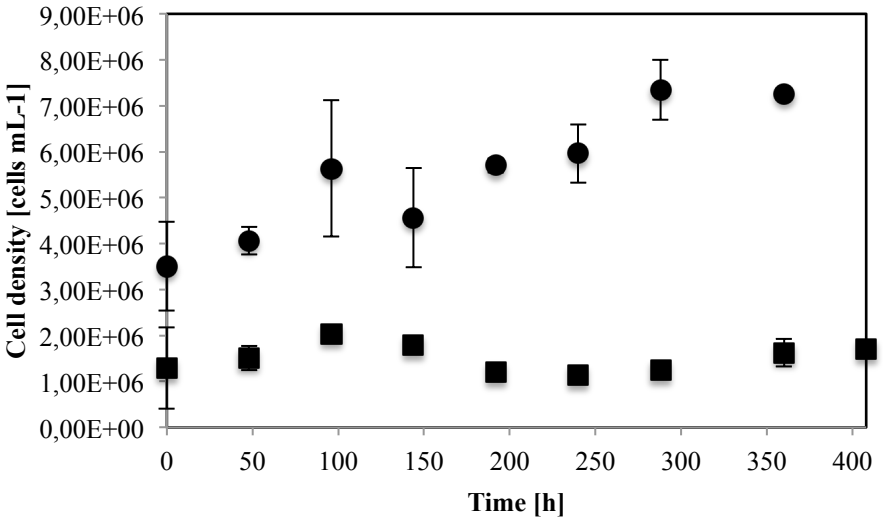

Figure 5: Cell density, non-aerated batch conditions (•), aerated batch conditions (■). Error bars (shown if larger than the symbols) represent standard deviations of duplicate assays.

Table 3: Initial and final biomass concentration and lipids content in non-aerated and aerated batch operation bioassays.

Parameter	Time	Unit	Non-aerated	Aerated
Biomass	Initial	mg L^{-1}	215.6	105.9
concentration	Final	mg L^{-1}	933.7	370.4
Lipids	Initial	%	11.8	8.6
content	Final	%	16.7	10.8